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Previews

The Long and Short of siRNAs

A recent work identifies a distinct class of siRNAs derived from transgenes and endogenous retroelements in plants (Hamilton et al., 2002). This class has slower electrophoretic mobility than previously characterized siRNAs and may play an important role in transgene-induced systemic silencing and in methylation of endogenous retroelement DNA.

Some ten years ago, an unexpected gene silencing response in transgenic plants was described. Within a set of plant lines harboring the same transgene sequence, a few lines fail to express the transgene and also fail to express transgene-homologous endogenes. From the combined efforts of many groups, we now know that this gene silencing phenomenon is due, at least in part, to aberrant expression of double-stranded RNA and that dsRNA-mediated gene silencing phenomena exist in probably all eukaryotes. Conserved mechanisms mediate dsRNA-triggered gene silencing, and these affect at least two different stages of mRNA production: transcription of mRNA (TGS) and posttranscriptional accumulation of mRNA (PTGS). In plants and other organisms, TGS is strongly correlated with the methylation of DNA sequences that correspond to the silenced locus, while PTGS is associated with degradation of cytoplasmic RNAs. Given that TGS, PTGS, and other silencing mechanisms might operate simultaneously in an organism (a possibility made more mechanistically comprehensible by new findings in Hamilton et al., 2002), the more operative and all-inclusive label "RNA silencing" is often applied to the phenomena.

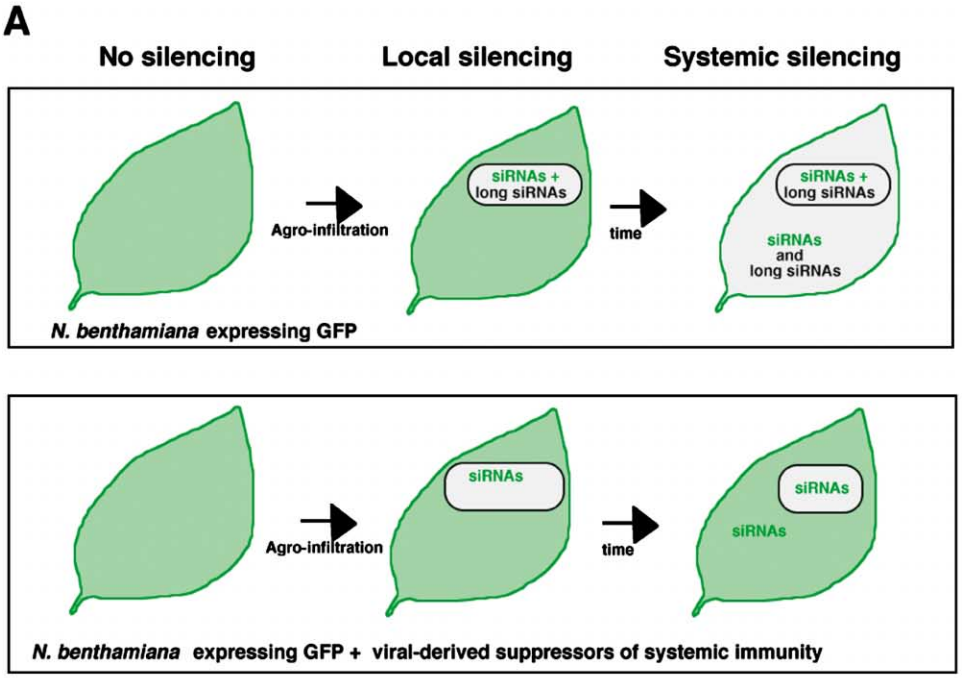
Many of the proteins that mediate RNA silencing are conserved (reviewed in Hannon, 2002). For example, Dicer homologs are found in all organisms. One of the many activities of Dicer enzymes is to manufacture small interfering dsRNAs (siRNA). siRNAs were first found in plants exhibiting transgene-mediated RNA silencing (Hamilton and Baulcombe, 1999), have subsequently been observed in diverse creatures that exhibit RNA silencing, and have the signature ends of an RNaseIII cleavage product as revealed by sequencing. Within cells, siRNAs are utilized by a multiprotein RNA silencing complex (RISC) to guide the sequence-specific degradation of mRNAs and are integral to the RNA silencing mechanism. Indeed, the introduction of siRNAs to cells and organisms is sufficient to induce RNA silencing phenotypes.

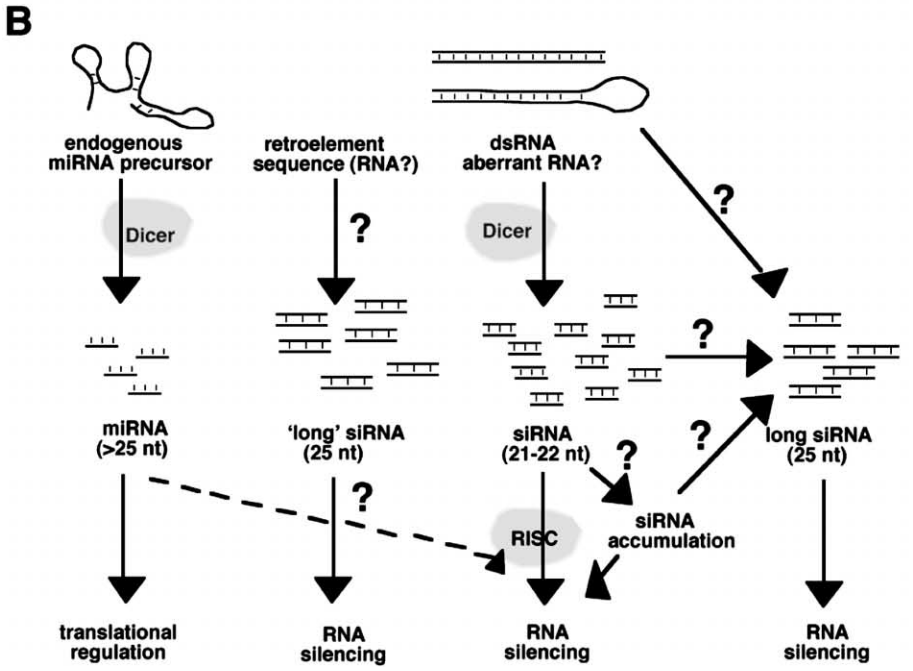
Some organisms respond systemically to localized delivery of RNA silencing triggers. This implies that a silencing trigger, or signal, can travel from the site of dsRNA introduction to remote cells. In plants, a mobile silencing signal can travel long distances and appears to take advantage of plasmodesmata for movement between cells and phloem for long-distance travels (reviewed in Mlotshwa et al., 2002). Little is known about the nature of the systemic silencing signal. Since sequence specificity is sustained during systemic silencing, one suspect for this signal is siRNA. Supportive of this notion is the observation that machine-synthesized siRNAs can mediate systemic silencing in plants (Klahre et al., 2002).

Clues to the identity of a systemic silencing signal in plants come from new findings by Hamilton et al. (2002). This group has now uncovered a second category of siRNAs, long siRNAs (25 nt), distinguishable by size from the 21–22 nt siRNAs class they had previously found (Hamilton and Baulcombe, 1999). In this latest study, a transient expression system based on *Agrobacterium tumefaciens* infiltration methodology was utilized to deliver a *gfp* RNA silencing signal to selected areas of transgenic, GFP-expressing *Nicotiana benthamiana* (see Figure, panel A). This method provides a good means to monitor both local and systemic silencing, as evidenced by loss of GFP fluorescence and reductions in *gfp* mRNA on Northern blots. Since local silencing precedes systemic silencing, the time after delivery, as well as the position of affected tissue relative to the delivery site, can be used to discriminate between locally and systemically affected tissues. Northern blots were used to detect differences in RNAs derived from these differently affected tissues. The analyses revealed siRNAs of two different classes, long (25 nt) and short (21–22 nt), in freshly treated tissue and in systemically affected plants.

RNA silencing confers protection against viral infection in plants (Waterhouse et al., 2001), and systemic signals undoubtedly play important roles in preventing spread of viruses. A mobile RNA silencing signal, generated in virus-infected cells and composed of viral sequences, may travel to uninfected tissues where it could be utilized to mount an RNA silencing response against invading viral genomes. Not surprisingly, viruses have evolved mechanisms to inhibit RNA silencing. Viral suppressor proteins can affect the systemic behavior of virus-derived and, in some instances, of transgene-derived RNA silencing signals. The Hamilton (2002) group took advantage of five viral suppressor proteins, each derived from a different virus, to study systemic silencing signals. Plants were coinfiltrated with a *gfp* silencing trigger along with DNAs encoding one of the viral suppressor proteins, and particular attention was paid to the effects on systemic silencing of the *gfp* target. Northern blots performed on locally and systemically affected tissues revealed a conspicuous absence of the long siRNA class of *gfp* RNAs in those plants that expressed suppressor proteins and were not systematically silenced. The consistent appearance of the long siRNA class in systemically affected tissue and its absence in nonaffected tissue suggests that this form of siRNA may have a specific role in systemic RNA silencing.

RNA silencing mechanisms also protect against transposition of mobile elements in the genome—mechanisms that may involve methylation of DNA sequences. These roles have not been fully investigated in plants. In Hamil-





Long siRNAs Associate with Systemic Silencing

(A) Induction of local and systemic silencing in plants using *Agrobacterium tumefaciens* infiltration of T-DNA sequences engineered to contain *gfp*. Silencing of *gfp* may spread to parts of plant outside the infiltration zone (top). Coinfiltration with a viral suppressor protein inhibits systemic silencing and correlates with an absence of long siRNAs (bottom).

(B) Simplified model showing steps in different, interrelated RNA silencing pathways that include production of miRNAs that regulate RNA translation (reviewed in Zamore, 2002), siRNAs that regulate RNA accumulation, and the newly described class of long siRNAs. The right half depicts possible synthesis routes of long siRNAs: long siRNAs might arise from the initial silencing molecule by a nonstandard Dicer cleavage event or by a Dicer paralog cleavage (downward arrow), from postcleavage modification of a siRNA (horizontal arrow), or from modification of secondary siRNAs that may accumulate as a result of an amplification event (upward arrow). These models may not be mutually exclusive, since the long siRNA class of molecules has not yet been demonstrated to be homogeneous in form.

ton et al. (2002), the authors used Northern blots of wild-type *Arabidopsis* and *Nicotiana* to see whether small RNAs for endogenous retroelements could be found. Interestingly, small dsRNAs were found and these corresponded to the long siRNA class only. (Long siRNAs may be produced from endogenous sources other than

retroelements—Llave et al. [2002] depict similarly sized siRNAs from wild-type *N. benthamiana* tissue [see Figure, panel B].) Unfortunately for the plant, Hamilton et al. (2002) found that the constitutive presence of this long siRNA species failed to direct the degradation of mRNAs harboring a section of retroelement-derived sequence, implying that long siRNAs have no role in targeting mRNA degradation.

Retroelement-derived long siRNAs were also assayed in *Arabidopsis* mutants with defects in RNA silencing. Of all the mutants tested, only one, *sde4*, inhibited accumulation of retroelement long siRNAs while the levels remained unchanged in other silencing-defective mutant backgrounds. They also observed that methylation of retroelement DNA was absent in *sde4* mutants, but was not inhibited in other silencing-defective mutants. This is a surprising result since reduced methylation of transgene sequences had previously been observed in all of these mutants, including *sde4* (Dalmay et al., 2000). The combined results correlate long siRNAs, methylation of DNA sequences, and *sde4* activity and suggest roles for these components in prevention of transposition; however, it remains to be seen whether increased retroelement transcription and mobilization occurs in *sde4* mutants. The identity of *sde4* may help explain how RNA silencing mechanisms can have differential effects on transgene versus retroelement sequences.

These latest results find an association of long siRNAs and short siRNAs together in locally silenced tissue, a correlation of long siRNAs to systemically silenced tissue, and a correlation of long siRNAs to retroelement DNA methylation in a *sde4*-dependent manner, but no correlation of long siRNAs with mRNA degradation. Questions remain as to the nature of the *sde4* gene and to the nature of the long siRNAs. Can the two different activities associated with long siRNAs (systemic silencing and DNA methylation) be attributed to the same molecule? The electrophoretic band corresponding in size to the long siRNAs may contain multiple modified or altered RNAs. It would be interesting to learn the sequences of long siRNAs—to determine whether they are composed primarily of promoter or intron sequences, for example. Questions arise as to the origins of long siRNAs. Are long siRNAs a product of Dicer cleavage, a product of a related enzyme (more than one Dicer exists in *Arabidopsis* [Jacobsen et al., 1999]), a postcleavage modification of a Dicer 21–22 nt siRNA, or a precleavage modification of the intact silencing trigger? Modifications that might lead to slower electrophoretic mobility include methylation, dephosphorylation, and nucleoside addition. In *Drosophila* extracts, some cytoplasmic siRNAs are not bound by a RISC complex (Nykanen et al., 2001). Could uncomplexed siRNAs be converted into long siRNA in organisms capable of systemic silencing? Are long siRNAs excluded from RISC? The findings related to endogenous retroelement forms suggest that long siRNAs have a role in the nucleus and raise additional questions. Is retroelement transcription required for the continuous presence of these long siRNAs? If long siRNAs are involved in a mechanism to prevent retroelement expression, why then do long siRNAs persist in wild-type plants? An enticing possibility arising from these studies is that a PTGS event may give rise to a secondary TGS signal that can act systemically.

While much remains to be done, the work by Hamilton et al. greatly expands the possibilities for identification of novel RNA silencing components and highlights the complexity of RNA silencing. Further examinations should provide new insights into processes that drive systemic RNA silencing and systemic protection from viral infections.

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Selected Reading

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Measuring the Immeasurable

Many bacterial pathogens turn on virulence genes at host body temperature. In the September 6, 2002, issue of *Cell*, Johansson et al. show that the *Listeria monocytogenes* thermosensor is an RNA structure in the 5′ untranslated region of the mRNA for the virulence-activating transcription factor PrfA. The stem-loop structure blocks translation initiation at 30°C but melts away at 37°C.

Organisms have evolved a bewildering array of sensory systems to interpret the world around them and regulate their most basic behaviors in response. At the cellular level, these pathways are all based on sensitive molecular monitors that can transduce a tiny stimulus into a behavior-modifying impulse. Some environmental cues,